Table 2

Distribution of mollusks on 12 *Diopatra* tube-caps from each site.

| Site | Number of individual mollusks per tube-cap | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
| Panama | | | 3 | 3 | 2 | | 2 | 1 | | 1 |
| Assateague | 6 | 2 | 3 | | | | 1 | | | |
| Woods Hole | 3 | 6 | 1 | 1 | 1 | | | | | |

and chitons were present, with gastropods being the most abundant, comprising 57% of the species and 66% of the individuals.

These results show that the crevices formed by the shells, shell fragments, and other debris incorporated into *Diopatra* tube-caps by their builders offer a previously unrecognized substrate for mollusks. It is particularly interesting that almost half of the species represented—all the *Crepidula* spp., *Theodoxus luteofasciatus, Anachis* sp., *Sphenia fragilis,* the chiton, *Crucibulum* sp., *Notoacmaea cf. subrotundata,* and *Seila adamsi*—are hard-bottom organisms. In the broad expanse of sand flats, *Diopatra* tube-caps may therefore represent a valuable resource, especially for those smaller species that could attain sexual maturity while living on this space-limited substrate. How effective tube-caps are as possible refuges from predation (small mollusks on sand flats can be subject to heavy predation pressure, *e.g.,* DUDLEY, 1980) or as long-term settlement sites for young individuals of species such as *Mercenaria mercenaria* that can attain large adult sizes, are questions to be resolved by an experimental approach. In view of the apparently widespread use of tube-caps by mollusks that this study documents such questions are worthy of investigation.

## Acknowledgments

We thank Erik Rensberger, Kendra Richardson, Barbara Schuel, Hilary Specht, and Amber Ulbrich for helping us gather data; Dr. and Mrs. Paul Wheeler for the use of their beach to collect specimens; and the staff at the Woods Hole Marine Biological Laboratory for the use of the library.

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# Dietary-Induced Hyperlipidemia in *Biomphalaria glabrata* (Gastropoda)

by
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## Introduction

The planorbid snail *Biomphalaria glabrata* (Say, 1818) is an intermediate host of the medically important trematode *Schistosoma mansoni,* and has been used for numerous physiological and biochemical studies. Relatively little information is available on lipids in this snail, although a recent study examined lipids in fed and starved *B. glabrata* (DUNCAN *et al.,* 1987).

Information on dietary-induced hyperlipidemia in this snail is not available. Unpublished studies in our laboratory show that *Biomphalaria glabrata* fed a high lipid diet, *i.e.,* hen's egg yolk, ingest and utilize the material, grow, and lay eggs. The purpose of this study is to determine if snails fed hen's egg yolk elevate their lipids compared to snails fed leaf lettuce. *Biomphalaria glabrata* may serve as a useful invertebrate model to study dietary-induced hyperlipidemia in humans.

## Materials and Methods

Snails, 10 ± 2 mm in shell diameter, were removed from stock cultures, placed in artificial spring water (DUNCAN *et al.,* 1987) and fed boiled hen's egg yolk (experimentals) or leaf lettuce (controls) *ad libitum.* Food and water were changed every other day, and some snails on experimental and control diets were removed for examination 1 and 2 weeks after the cultures were initiated.

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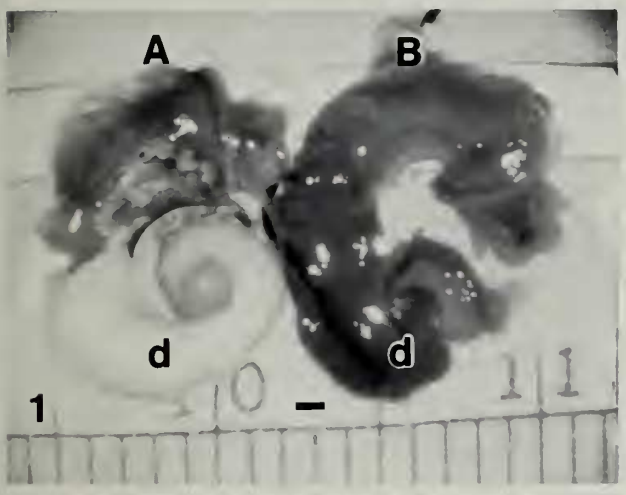


Figure 1

A photograph of live *Biomphalaria glabrata* snails fed hen's egg yolk (A) or leaf lettuce (B) for 2 weeks. Shells were removed to expose the body. The digestive gland-gonad complex (d) is white in the yolk-fed snail and green-brown in the lettuce-fed snail. Scale bar = 1 mm.

Two groups of 10 snails each, maintained for 1 or 2 weeks on either a yolk diet or lettuce diet, were analyzed gravimetrically. The whole snail (with shell) was weighed and then extracted with 3 mL of chloroform/methanol (2:1). Non-lipid contaminants were removed with the Folch wash (0.88% KCl), and the lipid extract of each snail was dried under nitrogen and weighed to determine the percentage of total lipid.

For most chromatographic analyses, one or two snails were treated as mentioned for the total lipid gravimetric analysis. For TLC studies the snail lipid extract was spotted on individual lanes in the preadsorbant area of a 20 × 20-cm precoated silica gel plate (Whatman LK6DF, Whatman Inc., Clifton, New Jersey). On the same plate was spotted 3 μL of the neutral lipid standard 18-4A (Nu-Chek Prep, Elysian, Minn.), consisting of 0.2 μg/μL each of cholesterol, oleic acid, triolein, methyl oleate, and cholesteryl oleate. Plates were developed 12 cm past the origin in a saturated glass rectangular tank with petroleum ether-diethyl ether-acetic acid (80:20:1). The lipids were detected by dipping the plates in 5% phosphomolybdic acid in ethanol and heating them in an oven at 100–120°C for approximately 5–10 min. The lipids appeared as blue-gray spots against a yellow background. Relative amounts of lipids were estimated by visual comparison of spot intensities between sample and standard chromatograms.

For capillary gas chromatography the lipid extracts of six snails fed lettuce and six snails fed yolk for 1 week were separated on preparative silica gel plates (Whatman PK1F, 1-mm layer thickness, Whatman Inc., Clifton, New Jersey) developed for 12 cm in petroleum ether-diethyl ether-acetic acid (80:20:1). Bands were scraped from the plate, packed in a microcolumn, and the sterols eluted with chloroform (Fried & Sherma, 1986). The sample was dried and reconstituted to 10 μg/μL with chloroform-methanol (2:1).

The sterols were analyzed by injecting 1 μL of sterol sample and standards (1 μg/μL solutions) into a Hewlett-Packard H-P 5890 gas chromatograph equipped with an H-P 3992A integrator/recorder, flame ionization detector, and a 15-m fused silica column (SPB-1) operated with a 1/30 split injection ratio and 270°C isothermal temperature for 25 min. Retention times of peaks in the samples were compared to those of standards for identification.

Table 1

TLC analysis of neutral lipids of whole snails fed lettuce (L) or yolk (Y) for 1 or 2 weeks.

| Lipid classes | $R_f$ values* | Week 1 | | Week 2 | |
|---|---|---|---|---|---|
| | | L | Y | L | Y |
| Free sterols | 0.18 | 2 | 2 | 2 | 2 |
| Free fatty acids | 0.30 | 0 | 1 | 0 | 1 |
| Triacylglycerols | 0.70 | 1 | 3 | 1 | 3 |
| Sterol esters | 0.94 | 1 | 2 | 1 | 2 |

Key: none = 0; trace = 1; moderate = 2; heavy = 3.
* Determined for 20 × 20-cm silica gel Whatman LK6DF plates developed for 12 cm from the origin in 100 mL of petroleum ether-diethyl ether-acetic acid (80:20:1) at 22–24°C.

For lipid histochemistry, four yolk-fed and four lettuce-fed snails, maintained on the diets for 2 weeks, were sectioned at 10 μm on a cryostat, and stained with Oil Red O (Lillie, 1944).

## Results and Discussion

The yolk-fed snails developed a white digestive gland-gonad complex while that of lettuce-fed snails remained green-brown (Figure 1). Regardless of the diet more than 90% of the snails were alive 2 weeks after the experiments were initiated.

Total lipid of whole yolk-fed *Biomphalaria glabrata* was compared with that of lettuce-fed snails. The yolk-fed snails ($n$ = 10) had an average percent lipid of 2.53 ± 0.14, whereas the lettuce-fed snails ($n$ = 10) had an average percent lipid of 1.68 ± 0.14. The increase in lipid occurred mainly in the digestive gland-gonad complex.

TLC showed a marked increase in the triacylglycerol fraction of yolk-fed snails at 1 and 2 weeks and a less marked increase in the free fatty acid and sterol ester fractions; an increase in the free sterol fraction was not apparent in yolk-fed snails (Table 1). When the sterol fraction was analyzed by GLC it was found, for both lettuce-fed and yolk-fed snails, that cholesterol was the major fraction with other sterols being present in smaller amounts. Sterols in both snails were campesterol, stigmasterol, and β-sitosterol. The yolk-fed snails in addition had desmosterol, coprostanol, and ketocholesterol.

The digestive gland-gonad complex of yolk-fed snails contained abundant lipid droplets stained with Oil Red O ranging in size from 20 to 40 μm. The same complex of lettuce-fed snails contained relatively few lipid droplets ranging in size from 5 to 10 μm.

## Acknowledgment

We thank the Dow Chemical Co. Foundation, Midland, Michigan for supporting the undergraduate research of S.S.

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## Range Correction for *Agaronia propatula* (Conrad, 1849)

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LOPEZ *et al.* (1988) report *Agaronia propatula* (Conrad, 1849) from Bahia de los Angeles, Baja California. The shells were actually collected by me near the type locality of *Epitonium textimatum* DuShane, 1977, at Playa de los Angeles, Tenacatita Bay, Jalisco, Mexico (see map in DUSHANE, 1977).

The correct range for the species should be from Jalisco, Mexico, to Panama.

Literature Cited

DUSHANE, H. 1977. *Epitonium textimatum*, a new gastropod from the west coast of Mexico. Nautilus 91:89–91.

LOPEZ, A., M. MONTOYA & J. LOPEZ. 1988. A review of the genus *Agaronia* (Olividae) in the Panamic Province and the description of two new species from Nicaragua. Veliger 30: 295–304.

## Worldwide Chiton Collection Available for Loan

A collection of chiton specimens, which previously formed a part of the collection of Dr. Ian McTaggart-Cowan, has been catalogued at the Royal British Columbia Museum. Many worldwide localities are represented, although most specimens come from British Columbia and the western coast of North America. Specimens are available for loan to scientists interested in studying the systematics, distribution, or taxonomy of this molluscan group. Most specimens are fluid preserved, although some are dry dorsal plates only. The collection contains 19 paratypes from 4 locations (Peden & Green, 1981, *Syesis* 14:155–162).

Requests for further information regarding this collection should be directed to:

Grant Hughes, Chief, Biological Collections, or
Philip Lambert, Head, Invertebrates
Royal British Columbia Museum
675 Belleville Street
Victoria, British Columbia
Canada V8V 1X4

## International Commission on Zoological Nomenclature

The following Opinion of potential interest to our readers has been published by the ICZN in the *Bulletin of Zoological Nomenclature,* volume 45, part 3, on 23 September 1988:

Opinion No. 1502. *Conus fergusoni* G. B. Sowerby III, 1873 (Mollusca, Gastropoda): specific name conserved.

The following application has been published on 16 December 1988 in volume 45, part 3 of the *Bulletin of Zoological Nomenclature.* Comment or advice on this application is invited for publication in the *Bulletin* and should be sent to the Executive Secretary, ICZN, British Museum (Natural History), Cromwell Road, London SW7 5BD, U.K.

Case 2643. *Iphinoe* Bate, 1856 (Crustacea, Cumacea): proposed conservation.

Conservation of the generic name *Iphinoe* Bate, 1856, of cumacean crustaceans involves the suppression of the gastropod name *Iphinoe* H. & A. Adams, 1854.